Motor Cooperativity and Motor Regulation

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How transport of subcellular commodities is regulated along intracellular transport pathways is a fundamental question in biology that has broad implications. Important steps have been taken towards understanding the properties of the molecular motors that drive intracellular transport processes. Yet, it has become increasingly clear that macromolecular complexes composed of multiple transport-related proteins commonly constitute the relevant transport 'machinery' of cells, as opposed to individual motor molecules. Herein, we describe new methods to examine the molecular organization and dynamic function of these important functional motifs and discuss how their collective behaviors can influence current notions of intracellular transport regulation.

***Keywords* — Intracellular Transport, Cooperativity, Macromolecular Complexes, Motor Regulation, Synthetic Biology.**

I. Introduction

THE transport of subcellular objects by the mechanochemical proteins kinesin, dynein and myosin constitutes a vital component of cellular physiology [1]. These proteins are molecular machines that harness the free energy from ATP hydrolysis to produce directed motions and the forces required to propel a wide variety of subcellular commodities (organelles, vesicles, protein signaling complexes, etc.) along a cell's cytoskeletal filaments. Since different types of motor proteins transport their cargo towards different intracellular destinations, molecular motors are directly responsible for establishing, maintaining, and regulating the internal organization of cells.

Although our understanding of motor mechanochemistry remains incomplete, the structural, biochemical and biophysical properties of many molecular motors have been studied in extreme detail. Yet, significant challenges remain to determine how motors actually function in living cells. These problems are often confounded by interactions between motors and other non-motile proteins that contribute to intracellular transport pathways. Many motors are known to associate with diverse sets of non-motile adaptor proteins and/or accessory factors in a combinatorial fashion. There is also growing evidence that a variety of intracellular transport processes entail the collective function of motor molecules, whereby several motor proteins work together in order to transport a cargo particle as a group [2-4]. Importantly, these multi-motor interactions can confer mechanical properties to a protein complex that are not found with single motor molecules. In many ways, this circumstance makes motor-protein complexes important regulatory motifs of the cell.

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II. Methods

We have established new biosynthetic routes to create structurally defined systems of interacting motor molecules whose collective dynamics can be investigated both *in vitro* and *in vivo* using single-molecule microscopy techniques, specifically single-particle tracking [3] and optical trapping [4]. Our biosynthetic tools allow key structural parameters of motor assemblies to be deterministically controlled (i.e., cargo size, motor number, elasticity of motor interconnects, and the intermotor spacing). In turn, these synthetic handles allow us to reliably interpret complex dynamic behaviors of multi-unit motor systems, and hence, investigate critical aspects of motor regulation. For example, in a system of two-interacting kinesin-1 motors both single particle (single-assembly) tracking and optical trapping experiments reveal that motors have a strong tendency to interfere with one another during collective transport. While increased force production and long cargo run lengths compared to single motor molecules are observed, motor interference, or negative cooperativity, increases the probability that a single motor molecule within an assembly detaches from the microtubule. This type of self-organized attenuation of mechanical microstates in the assembly should influence the motile responses of motor complexes to many biochemical regulatory factors and mechanical challenges along intracellular transport pathways.

III. Conclusion

The creation of structurally-defined complexes of motor molecules now provides key avenues to deconvolve complex transport behaviors of motor proteins in cells. By analyzing the motions of multi-unit motor assemblies and their distributions of mechanical microstates with 'single-molecule' resolution, critical aspects of collective motor function are revealed that challenge current notions of intracellular transport regulation.

References

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